



Application of a green manure and green manure composted with beet vinasse on soil restoration: Effects on soil properties

M. Tejada ^{a,*}, J.L. Gonzalez ^b, A.M. García-Martínez ^c, J. Parrado ^c

^a Departamento de Cristalografía, Mineralogía y Química Agrícola, E.U.I.T.A. Universidad de Sevilla, Crta de Utrera km. 1, 41013 Sevilla, Spain

^b Departamento de Química Agrícola y Edafología, Universidad de Córdoba, Campus de Rabanales, Edificio C-3, Crta N-IV-a, km. 396, 14014 Córdoba, Spain

^c Departamento de Bioquímica, Toxicología y M.L. Facultad de Farmacia, Universidad de Sevilla, C/Prof. García Gonzalez 2, 41012 Sevilla, Spain

Received 15 January 2007; received in revised form 5 September 2007; accepted 10 September 2007

Abstract

Beet vinasse (BV), a green manure constituted by *Trifolium pratense* L. uncomposted (TP) and composted with beet vinasse (at 1:1 rate, (TP + BV)1, and 2:1 rate, (TP + BV)2) at 10 t organic matter ha⁻¹ rate were applied during a period of four years for purpose of restoration of a Xelloric Calciorthid located near Seville (Guadalquivir Valley, Andalusia, Spain). The effect on the plant cover, soil physical (structural stability and bulk density), chemical (exchangeable sodium percentage), and biological properties (microbial biomass, soil respiration and enzymatic activities such as dehydrogenase, urease, β -glucosidase, phosphatase and arylsulfatase) were determined. The application of BV had a detrimental impact on soil physical (structural stability decreased 16.5% and bulk density increased 18.7% respect to the control soil), chemical (exchangeable sodium percentage increased 87.3% respect to the control soil), and biological properties (microbial biomass, soil respiration, and dehydrogenase, urease, β -glucosidase, phosphatase and arylsulfatase activities decreased by 53.5%, 24.5%, 27.8%, 15%, 39.7%, 42.7%, and 65.6%, respectively with respect to the control soil), probably because high quantities of monovalent cations (Na principally) were introduced into the soil by the vinasse, thus destabilizing its structure. The application of TP had a positive impact on soil physical (structural stability increased 5.9% and bulk density decreased 6.1% respect to the control soil), and biological properties (microbial biomass, soil respiration, and dehydrogenase, urease, β -glucosidase, phosphatase and arylsulfatase activities increased by 66.3%, 45.6%, 97.7%, 98.9%, 97.7%, 87.2%, and 89.4%, respectively with respect to the control soil). However, when BV was co-composted with a green manure, principally at a 2:1 rate, the resulting compost had a positive effect on soil physical (structural stability increased 10.5% and bulk density decreased 13.5% respect to the control soil), and biological properties (microbial biomass, soil respiration, and dehydrogenase, urease, β -glucosidase, phosphatase and arylsulfatase activities increased by 68.9%, 46.2%, 97.5%, 98.4%, 99.1%, 90.5% and 91.6%, respectively with respect to the control soil). After four years, the percentage of plant cover decreased 64.3% in the BV-amended plots respect to the control soil, whereas increased 82.8%, 81.6% and 81% in the (TP + BV)2, (TP + BV)1 and TP treatments, respectively. While the application of BV deteriorates the soil and therefore does not contribute to its restoration, the application of TP, and BV composted with TP protects the soil and will contribute to its restoration.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Soil restoration; Beet vinasse; Green manure; Green manure composted with beet vinasse

1. Introduction

One of the major environmental concerns is soil degradation. Inappropriate technologies have resulted in soil

quality deterioration, leading to soil organic matter losses and structure degradation, affecting water, air and nutrients flows, and consequently plant growth (Golchin et al., 1995; Tejada et al., 2006b). For this reason, the application of organic wastes with a high organic matter content, such as fresh and composted urban wastes (Ros et al., 2003), shredded and composted plant materials derived from

* Corresponding author.

E-mail address: mtmoral@us.es (M. Tejada).

municipal landscape (Walker, 2003), and cotton gin compost and poultry manure (Tejada et al., 2006b) to semiarid soils has become a common environmental practice for soil restoration, maintaining soil organic matter, reclaiming degraded soils, and supplying plant nutrients.

Organic by-products originating from industrial processes represent an important source of nutrients, especially for organic fertilization. In this respect, beet vinasse, a final by-product of the sugar industry, is a product of great interest because of its organic matter content (398 g kg⁻¹), N (32.5 g kg⁻¹) and K (126 g kg⁻¹) concentrations. Sugar beet is processed to produce crystalline sugar, pulp and molasses, the last being fermented to produce alcohol. After removal of the alcohol by distillation the remaining material is known as vinasse. In SW Spain, the annual production of beet vinasse is about 5×10^3 Mg (Madejon et al., 2001).

However, beet vinasse presents a high Na concentration (21 g kg⁻¹). This aspect is the main responsible for the deterioration of soil physical, chemical and biological properties after the addition of beet vinasse to soil under dryland conditions, favouring soil erosion as well as a decrease in soil microbial biomass and in crop productivity (Tejada and Gonzalez, 2005, 2006a). Some authors have suggested that the problems associated with the addition to soil fresh residues (such as beet vinasse) may be overcome by co-composting with solid agricultural wastes (Madejon et al., 2001; Diaz et al., 2002).

The application of green manures to soil is considered a good management practice in any agricultural production system because stimulate soil microbial growth and activity, with subsequent mineralization of plant nutrients (Eriksen, 2005), and therefore increase soil fertility and quality (Doran et al., 1988). Leguminous and non-leguminous plants are used as green manures. Leguminous green manures can fix large quantity of atmospheric N₂ and can provide useful amounts of organic matter on soil. Non-leguminous green manures only can increase the organic matter in soil and do not fix atmospheric N₂. For this reason, beet vinasse may be overcome by co-composting with green manures.

Physical and chemical properties have been used to evaluate the effects of the application of different sources of organic matter on soil during long-term experiments (Tejada and Gonzalez, 2004). However, such properties change very slowly and need many years to provide any significant results. In contrast, microbiological and biochemical properties are very responsive and provide immediate and precise information on small changes occurring in soil (Dick and Tabatabai, 1993). In fact, they may also indicate the soil's potential to sustain microbiological activity (Paul and Clark, 1989).

In view of the above, the objective of this study was to evaluate the effects of using fresh beet vinasse, green manures and beet vinasse composted with green manures as a bulking agent, as soil amendments at different rates on soil restoration, comparing some physical (soil structural sta-

bility and soil bulk density), chemical (exchangeable sodium percentage) and biological soil properties (soil respiration, soil microbial biomass and soil enzymatic activities) in a semiarid Mediterranean agro-ecosystem.

2. Methods

2.1. Site and properties of organic wastes

The study was conducted from October 2001 to October 2005 near Seville (Guadalquivir Valley, Andalusia, Spain) on a Xerollic Calciorthid (Soil Survey Staff, 1987) with a 2% slope. The general properties of this soil (0–25 cm) are shown in Table 1. Soil pH was determined in distilled water with a glass electrode (soil:H₂O ratio 1:1). Soil electric conductivity was determined in distilled water with a glass electrode (soil:H₂O ratio 1:5). Soil texture was determined by the Robinson's pipette method (SSEW, 1982) and dominant clay types were determined by X-ray diffraction. Soil Kjeldahl-N was determined by the Kjeldahl method (MAPA, 1986). Total CaCO₃ was measured by estimating the quantity of the CO₂ produced by HCl addition to the soil (MAPA, 1986). Soil organic carbon was determined by oxidizing organic matter in soil samples with K₂Cr₂O₇ in sulphuric acid (96%) for 30 min, and measuring the concentration of Cr³⁺ formed (Sims and Haby, 1971).

The organic wastes applied were: fresh non-depotassified beet vinasse (BV), a green manure composted and constituted by leguminous plants (*Trifolium pratense*, L.) (TP), and a compost obtained by mixing of TP and BV at a 1:1 rate [(green manure:beet vinasse) (weight:weight)] and 2:1 rate [(green manure:beet vinasse) (weight:weight)].

Plant residues were composted. Prior to composting, the residues were crushed using a shredder (Bione sgon 2.0, Sandri Garden srl). TP residues were composted in trapezoidal piles (2 m high by 2 m width by 3 m bug) and under aerobic conditions. The compost was turned once a week during the first month and every other week thereafter in order to maintain an aerobic environment in the pile. During the thermophilic phase, the piles were watered regularly

Table 1
Initial soil characteristics and standard error in parenthesis (data are the means of four samples)

pH	7.6 (0.14)
Electrical conductivity (dS m ⁻¹)	0.23 (0.04)
Clay (g kg ⁻¹)	313 (15)
Silt (g kg ⁻¹)	259 (22)
Sand (g kg ⁻¹)	428 (31)
Texture	Clay loam
Dominant clay types	Illite, illite-montmorillonite (interstratified)
CaCO ₃ (g kg ⁻¹)	351 (25)
Kjeldahl N (g kg ⁻¹)	0.9 (0.03)
Total C (g kg ⁻¹)	6.3 (0.09)
C/N ratio	7 (3)

to maintain moisture contents at around 55%. To assure an adequate composting process, 50–60% is the recommended range for moisture content (McKinley et al., 1985). The composting process was carried on 179 days, when the C:N ratio and the temperature had become constant.

The combination of TP + BV was composted under similar conditions to those previously described. The composting process lasted 190 days for the proportion 1:1 and 207 days for the proportion 2:1.

The general properties of the organic wastes at the end of the composting process are shown in Table 2. Organic matter content was determined by dry combustion method (MAPA, 1986). To determine humic and fulvic acids-C, organic waste was extracted with 0.1 M sodium pyrophosphate and 0.1 M sodium hydroxide at pH 13 (Kononova, 1966). The supernatant was acidified to pH 2 with HCl and allowed to stand for 24 h at room temperature. To separate humic acids from fulvic acids, the solution was centrifuged and the precipitate containing humic acids was dissolved with sodium hydroxide (Yeomans and Bremner, 1988). After the removal of humic acids, the acidic filtrate containing the dissolved fulvic acid fraction was passed through a column of XAD-8 resin. The adsorbed fulvic was then recovered by elution with 0.1 M NaOH, desalted using Amberlyst 15-cation-exchange resin, and finally freeze-dried. The carbon content of humic acid and fulvic acids was determined by the method of Sims and Haby (1971). For BV, inorganic soluble P (PO_4H_2^- principally) was determined by Willians and Stewart method, described by Gitian and Carballas (1976). For green manures and green manures + BV, inorganic soluble P were determined by Willians and Stewart method, described by Gitian and Carballas (1976) after nitric and perchloric acid digestion. For BV, K and Na were determined by atomic emission spectrometer, and for green manures and green manures + BV, K and Na were determined by atomic emission spectrometer after nitric and perchloric acid digestion.

2.2. Experimental layout and treatments

The experimental layout was in a randomized complete block design with five treatments and three replicates per treatment. The plot size was 5 m × 6 m. The treatments were the following: (1) Control soil, non-fertilized control

plot; (2) BV, fertilized with 25.12 t ha⁻¹ of BV (10 t organic matter ha⁻¹, fresh matter, 20100 l ha⁻¹); (3) TP, fertilized with 28.571 t ha⁻¹ of TP (10 t organic matter ha⁻¹, fresh matter); (4) (TP + BV)1, fertilized with 16.722 t ha⁻¹ of TP + BV at a 1:1 rate (TP: BV) (weight:weight) (10 t organic matter ha⁻¹, fresh matter); and (5) (TP + BV)2, fertilized with 13.423 t ha⁻¹ of TP + BV at a 2:1 rate (TP: BV) (weight:weight) (10 t organic matter ha⁻¹, fresh matter).

BV was mixed with 2000 l ha⁻¹ of water with the objective of decreasing the density of the byproduct and facilitating its handling in field. The organic wastes were applied to the soil surface at the same amount on 10 October 2001, 2 October 2002, 7 October 2003 and 6 October 2004, respectively. The quality of all organic wastes was the same during the experimental period. In this respect, organic wastes were kept in refrigeration 0 °C after their application the first year, so that there were no problems of mineralization of the organic compounds of these products.

2.3. Soil sampling and analytical determinations

Plant cover, or percentage of soil covered by the octagonal projection of the aerial part of each plant, was determined by the lineal intercept method (Canfield, 1941).

Soil samples (0–25 cm) were collected from each plot with a gauge auger (30-mm diameter) on 1 October 2002, 6 October 2003, 5 October 2004, and 8 October 2005, respectively. Three subsamples were collected from each plot. After air drying, the soil samples were ground to pass a 2-mm sieve and stored in sealed polyethylene bags at 4 °C until analysis.

Soil structural stability was determined by the Hénin and Monnier method (1956) and classified according to Baize criteria (1998). The aggregate size fraction <2 mm was used. The proportions (% w/w) of stable Ag, Ag_a and Ag_b aggregates (corresponding to untreated, alcohol-treated and benzene-treated aggregates, respectively) were calculated, and the instability index, Is, was obtained using the equation:

$$Is = \frac{(\% < 20 \mu\text{m}) \max}{\frac{Ag + Ag_a + Ag_b}{3} - 0.9(\% \text{ CS})}$$

Table 2

Characteristics of organic wastes used and standard deviation in parenthesis (data are the means of six samples)

	BV	TP	(TP + BV)1	(TP + BV)2
pH (H ₂ O)	4.8 (0.2)	6.2 (0.1)	5.3 (0.1)	5.6 (0.1)
Density (Mg m ⁻³)	1.25 (0.11)			
Organic matter (g kg ⁻¹)	398 (18)	350 (11)	598 (15)	745 (21)
Humic acid (g kg ⁻¹)	0.64 (0.17)	10.2 (0.1)	10.7 (0.2)	20.2 (0.3)
Fulvic acid (g kg ⁻¹)	81.0 (5.6)	34.8 (1.6)	65.6 (0.8)	85.3 (1.6)
Total N (g kg ⁻¹)	32.5 (2.4)	40.8 (1.7)	64.8 (1.2)	82.2 (1.9)
C:N ratio	5.9 (0.2)	8.8 (0.7)	27.4 (1.3)	30.3 (1.2)
P (g kg ⁻¹)	0.3 (0.1)	3.5 (0.4)	1.9 (0.2)	3.2 (0.7)
K (g kg ⁻¹)	126 (11)	10.1 (0.5)	66.4 (1.4)	83.5 (2.2)
Na (g kg ⁻¹)	21 (4)	0.12 (0.05)	10.1 (1.2)	6.8 (0.2)

where (% < 20 μm) max indicates the largest proportion of suspended particles <20 μm determined for the three samples treatments, and % CS is the largest proportion of coarse sand (the 0.2–2 mm fraction) forming part of the stable aggregates.

Soil bulk density was determined using the core method. The soil was weighed and dried at 105 °C for 48 h before determining bulk density as the ratio between soil dry weight and the ring volume, according to the official methods of the Spanish Ministry of Agriculture (MAPA, 1986).

The exchangeable sodium percentage (ESP) was also determined, using the formula

$$\text{ESP} = 100 \times \left(\frac{\text{Na}_x}{\text{CES}} \right)$$

where Na_x is the exchangeable sodium (cmol kg^{-1}) and CEC is the cation exchange capacity of the soil (cmol kg^{-1}). Exchangeable sodium (Na_x) was determined with 1 M ammonium acetate at pH 7 (Richards, 1954) and the cation exchange capacity was determined with 1 M ammonium chloride solution in ethanol/water (60:40 v/v) at pH 8.2 (Tucker, 1954). Extracted Na was determined by flame photometry.

Soil microbial biomass was determined using the CHCl_3 fumigation-extraction method (Vance et al., 1987). The activity levels of six soil enzymes were measured. Dehydrogenase activity was measured by reduction of 2-*p*-iodo-3-nitrophenyl 5-phenyl tetrazolium chloride to iodonitrophenyl formazan (García et al., 1993). Urease activity was determined by the buffered method of Kandeler and Gerber (1988) using urea as substrate. β -glucosidase activity was determined using *p*-nitrophenyl- β -D-glucopyranoside as substrate (Masciandaro et al., 1994). Alkaline phosphatase activity was measured using *p*-nitrophenyl phosphate as substrate (Tabatabai and Bremner, 1969). Arylsulfatase activity was determined using *p*-nitrophenylsulphate as substrate (Tabatabai and Bremner, 1970).

In the laboratory, and in the samples at the end of the experiment (8 October 2005), soil respiration for all treatments was measured by incubation for 3, 7, 15, 30, 60 and 90 days. Total C-CO₂ collected in the NaOH flasks was determined by the addition of an excess of 1.5 M BaCl₂ followed by titration with standardized HCl using a phenolphthalein indicator (Zibilske, 1994).

2.4. Statistical Analysis

Analysis of variance (ANOVA) was performed using the Statgraphics v. 5.0 software package (Statistical Graphics Corporation, 1991). ANOVA was based on the LSD criterion (least significant differences between means using Student's *t*), considering a significance level of $p < 0.05$ throughout the study. For the ANOVA analysis, the triplicate data were used for each fertilizer treatment and every experimental season; although in the tables the values that appear are the average of the triplicate. Data were sub-

jected to factor analysis using varimax rotated factor matrix.

3. Results

3.1. Soil physical and chemical properties

At the end of the experimental period, structural stability decreased in soils amended with BV but increased in soils amended with the others organic wastes (Fig. 1). In this respect, instability index increased 16.5% in BV-amended soils with respect to the control soil, whereas TP treatment increased structural stability 5.9%, and for TP + BV composts the highest structural stability was observed in the soils amended with the organic waste with a higher humic acid concentration, (TP + BV)2. Thus, soil structural stability increased 10.5% and 7.9% in soils amended with (TP + BV)2 and (TP + BV)1, respectively, with respect to the control soil. Statistical analysis showed differences between the treatments at the end of experimental period.

Soil bulk density increased 18.7% in BV-amended soils with respect to control soil. However, soil bulk density

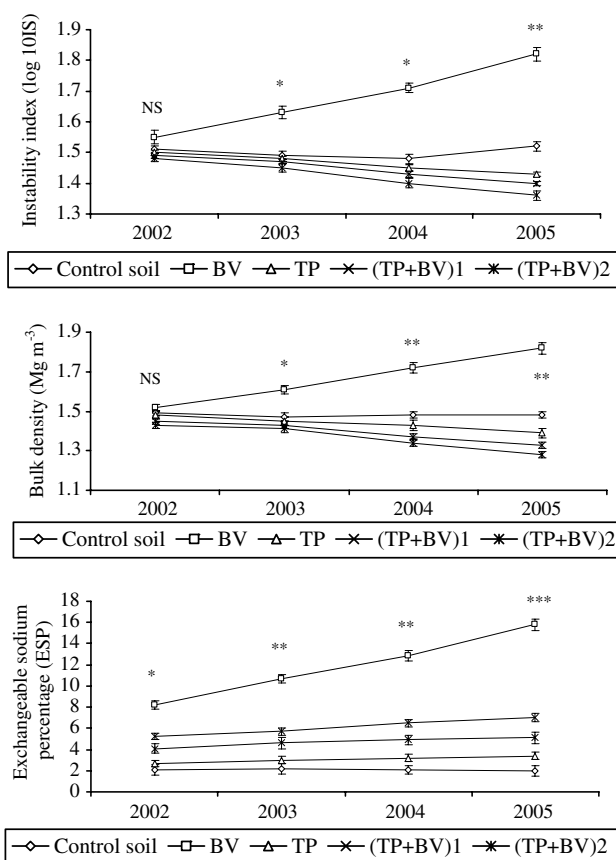


Fig. 1. Instability index (log 10 Is), bulk density (Mg m^{-3}), and exchangeable sodium percentage (ESP) (d), in soils to which organic wastes were applied. Error bars represent the standard error of means. Structural stability (log 10 Is) (Baize, 1988): “Very stable = <1.0”; “Stable = 1.0–1.3”; “Slightly stable = 1.3–1.7”; “Unstable = 1.7–2.0”; “Very unstable = >2.0” NS, *, **, ***: non-significant or significant at $p < 0.05$, 0.01 or 0.001, respectively.

decreased in the others amended soils. This decrease was higher in the soils amended with those organic wastes with a higher content in fulvic acids. In this respect and at the end of the experimental period, bulk density decreased 6.1%, 10.1% and 13.5% in soils amended with TP, (TP + BV)1, (TP + BV)2, respectively. Again, the statistical analysis showed differences between the treatments at the end of experimental period.

Exchangeable sodium percentage increased significantly in BV-amended soils when compared with the others organic waste amended soils during the experimental period. For BV amended soils ESP had reached critical sodicity values (ESP 15.8) mentioned by Richards (1954) at the end of the experimental period. However, for the others organic wastes amended soils, ESP did not reach the critical sodicity value of around 15 mentioned by Richards (1954). The control soil presented the lowest ESP value of all treatments assayed. The statistical analyses showed important significant differences for all treatments during the experimental period.

Because the same amount of organic matter was added to the soils, the variability in the results obtained in the soil physical and chemical properties must be due to the different chemical nature of the various organic wastes added to the soil (Table 2).

3.2. Soil biological properties

Fig. 2 shows the respiration (CO_2 evolved during 90 days incubation experiment) and microbial biomass values

in the soil during the experimental period. At first sight, the results for both parameters seem contradictory since the data for these parameters differ substantially for the same rate of organic matter applied to the soil.

At the end of experimental period, the soil microbial biomass and soil respiration values for BV-amended soils were different from the values obtained for soils amended with the others organic wastes; both parameters decreasing in the first case and increasing in the second. In this respect, soil respiration decreased 24.5% and soil microbial biomass decreased 53.5% in BV-amended soil respect to the control soil. Respect to the TP-amended soils and at the end of the experimental period, soil respiration and soil microbial biomass increased 45.6% and 66.3%, respectively, respect to the control soil. Respect to the green manures composted with BV, the highest respiration and microbial biomass was observed in the soils amended with the organic wastes with a higher fulvic acid concentration. In this respect, soil respiration was highest 85.2% and 83.3%, respectively in (TP + BV)2 and (TP + BV)1 respect to the control soil at the end of the experiment. Also, soil microbial biomass was highest 68.9% and 67.8%, respectively in (TP + BV)2 and (TP + BV)1 respect to the control soil at the end of the experiment. The statistical analyses showed important significant differences for all treatments during the experimental period.

The activity of the dehydrogenase enzyme was significantly inhibited at the end of the experimental period in the BV-amended soils (Fig. 2). However, it was significantly stimulated in the other treated soils. However, this

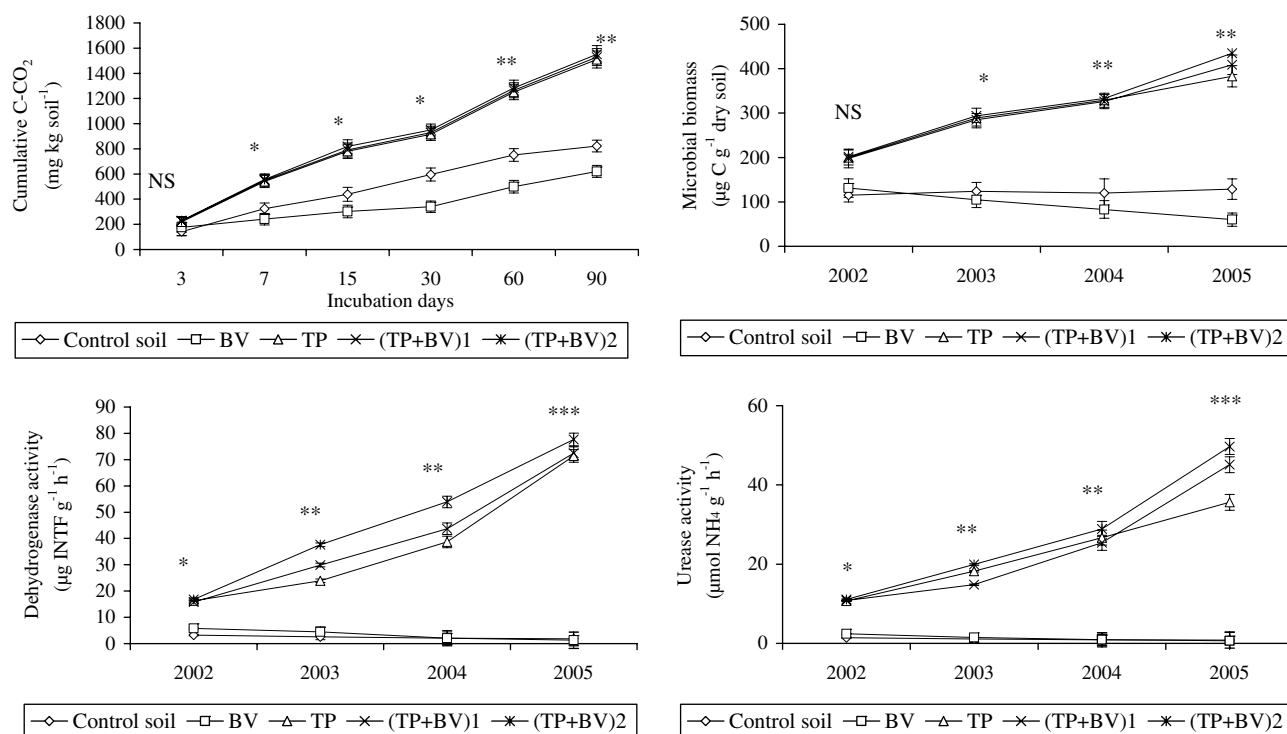


Fig. 2. Cumulative CO_2 -C, microbial biomass-C, dehydrogenase and urease activities in soils to which organic wastes were applied. Error bars represent the standard error of means. INTF: 2-*p*-iodo-3-nitrophenyl. NS, *, **, ***: non-significant or significant at $p < 0.05$, 0.01 or 0.001, respectively.

stimulation depends of the chemical composition of organic matter applied to the soil, highlighting a higher stimulation of this enzyme in soils amended with those organic wastes with a higher concentration of fulvic acids.

As in the case of dehydrogenase activity, urease, β -glucosidase, phosphatase and arylsulfatase activities was strongly inhibited by BV addition to the soils, whereas was stimulated by the addition to the soil of TP, (TP + BV)1 and (TP + BV)2 treatments (Figs. 2 and 3). Again, the stimulation of these enzymes depends of the chemical composition of the organic wastes. Again, the statistical analyses showed important significant differences for all treatments during the experimental period.

3.3. Plant cover

One year after the organic amendment, the treated plots were covered with spontaneous vegetation; *Borago officinalis*, *Chrysanthemum coronarium*, *Diplotaxis muralis*, *Lavatera cretica*, *Paronychia argentea*, *Trifolium angustifolium* and *Trifolium stellatum*, being the most abundant species. Fig. 4 shows the evolution of percentage of plant cover after the application of the organic wastes during the experimental period. After 4 years, the percentage of

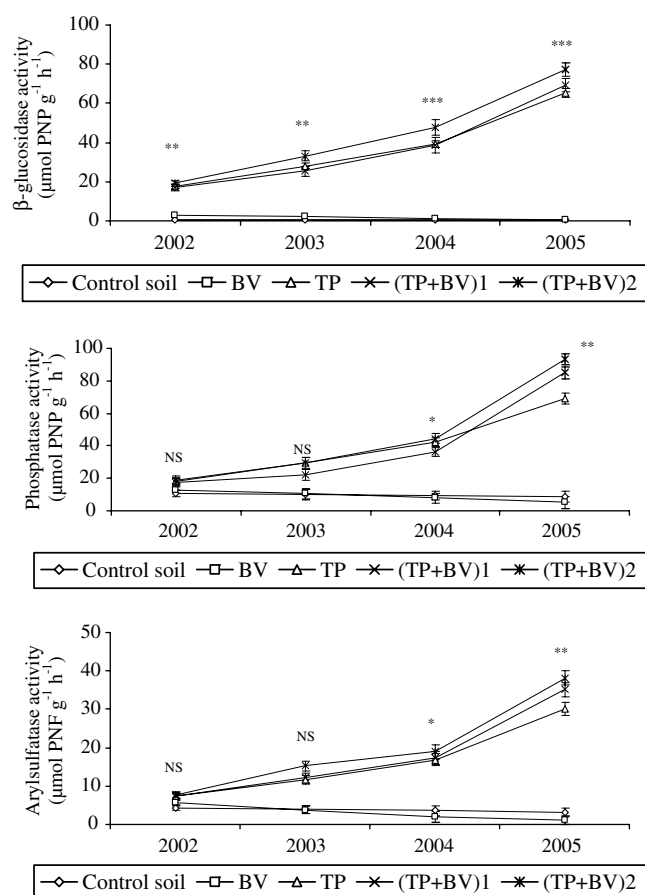


Fig. 3. β -Glucosidase, phosphatase, and arylsulfatase activities in soils to which organic wastes were applied. Error bars represent the standard error of means. PNP: *p*-nitrophenol; PNF: *p*-nitrophenyl NS, *, **, ***: non-significant or significant at $p < 0.05$, 0.01 or 0.001, respectively.

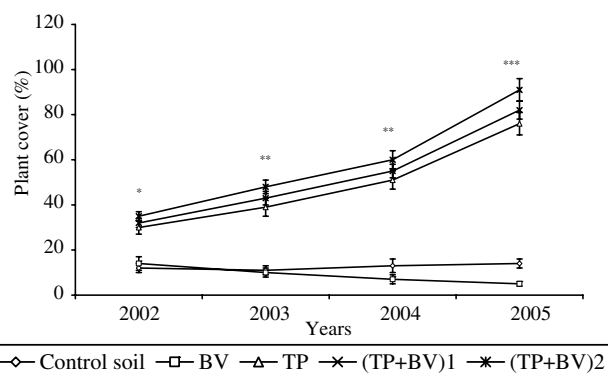


Fig. 4. Plant cover (%) in soils to which organic wastes were applied. Error bars represent the standard error of means. NS, *, **, ***: non-significant or significant at $p < 0.05$, 0.01 or 0.001, respectively.

Table 3

Factorial analysis for all parameters analyzed and for all treatments studied

	Eigenvalue	% Variance	Varimax rotated factor matrix	
			1	2
Instability index	7.679	76.789	−0.533	0.759
Bulk density	1.397	13.969	−0.545	0.761
ESP	0.564	5.639	0.097	0.845
Microbial biomass	0.179	1.795	0.904	−0.308
Dehydrogenase activity	0.084	0.839	0.974	−0.194
Urease activity	0.039	0.397	0.969	−0.202
Glucosidase activity	0.028	0.283	0.973	−0.203
Phosphatase activity	0.013	0.129	0.968	−0.133
Sulfatase activity	0.009	0.089	0.916	−0.148
Plant cover	0.007	0.071	0.944	−0.211

plant cover decreased 64.3% in the BV-amended plots with respect to the control soil, and increased in the other organic wastes amended plots. However, this increase depends of the chemical composition of organic matter applied to the soil. The percentage of plant cover increased respect to the control soil in following order: plot amended with (TP + BV)2 (82.8%) > plot amended with (TP + BV)1 (81.6%) > plot amended with TP (81.0%).

In order to predict the properties most decisively influencing soil remediation, variables were subjected to factorial analyses (Table 3). The factors selected were those having a greater-than-unity eigenvalue, and these factors were found to account for 90.758% of the overall variance. Varimax rotated factor matrix indicated two factors. The first factor was found to encompass the variables instability index (log 10 Is), bulk density and ESP, while the second factor encompassed the variables microbial biomass, enzymatic activities and plant cover.

4. Discussion

4.1. Soil physical and chemical properties

The results obtained for BV-amended soils are very different than obtained for the other organic waste amended soils.

For BV-amended soils, the organic amendment decreased soil structural stability and increased soil bulk density. This was especially evident at the end of experimental period. According with Tejada and Gonzalez (2005) and Tejada et al. (2006c), the negative effect of vinasse on soil structure may be due to its nature, since BV contains a high concentration of Na^+ . Pernes-Debuyser and Tessier (2004) and Bronick and Lal (2005) found that the Na^+ is a highly dispersive agent resulting directly in the breakup of aggregates. Exchangeable Na^+ in the soil solution and at the exchange sites contribute to repulsive charges that disperse clay particles. Exchangeable sodium percentage increased during the experimental period. According to Mamedov et al. (2002), this increase favours the dispersibility and aggregate disintegration in these soils. Also, BV is a waste with low humic acid-C and high fulvic acid-C concentrations. Piccolo et al. (1997) and Whalen et al. (2003) suggested that the aggregate stability is significantly correlated with humic but not to the fulvic acid concentration, because the humic acids are directly involved in the clay–organic complex formation, whereas the fulvic acids not. For this reason, the aggregates formed in BV-amended soils are not very stable (Tejada and Gonzalez, 2005; Tejada et al., 2006c).

TP and TP + BV treatments increased soil structural stability. These results are in agreement with those of Spaccini et al. (2004), Tejada and Gonzalez (2006a) and Tejada et al. (2006c) who found that organic matter acts as a cementing factor, necessary for flocculated soil particles to form stable aggregates. The highest values of structural stability were observed in TP + BV treatments followed by TP treatment. Probably the better behaviour of the TP + BV composts with respect to TP is due to their higher humic substance content, which decreases in the following order: (TP + BV)₂ > (TP + BV)₁ > TP. Chaney and Swift (1984) and Piccolo and Mbagwu (1990) suggested that the aggregate stability is significantly correlated with humic acid concentration, because the humic acids are directly involved in the clay–organic complex formation.

In addition, bulk density decreased as a result of the dilution of the denser soil mineral fraction and soil aeration increased because of the increase in soil porosity with the structural stability. This increase was especially evident for green manure composted with BV and at the end of the experimental period and was in line with Kay et al. (1997) and Tejada and Gonzalez (2006a) and Tejada et al. (2006c).

4.2. Soil biological properties

BV treatment decreased soil microbial biomass, soil respiratory activity and soil enzymatic activities. According to Tejada and Gonzalez (2005, 2006a) and Tejada et al. (2006c) the degradation of soil structure and increase of soil bulk density and ESP, can cause the development of soil anaerobic conditions and therefore a decrease in soil microbial biomass, soil respiration and soil enzymatic

activities. According to Tate (2002) oxygen concentration in soil can affect the metabolic status of the enzyme-producing cells. Disruption of soil aggregates alters negatively the oxygen diffusion rate from the atmosphere above the soil into the soil matrix as well as the rate of its consumption.

Our results showed that the soil in which a decrease in soil microbial biomass was observed, showed a high soil instability index ($\log 10 \text{ Is}$), which is perhaps due to the addition of large amounts of this organic amendment. Several studies have indicated that soil microbial processes are directly and indirectly influenced by soil structure; for example, the inverse relationship between ESP and soil organic matter content has long been known (Aylmore and Sills, 1982). The presence of small pores reduces accessibility of organic materials to decomposers, leading to the physical protection of C and a reduction in N mineralization (Van Veen and Kluikman, 1990). The spatial distribution of microbes and soil mesofauna has been shown to be partially associated with the size distribution of aggregates (Jastrow and Miller, 1991). Obviously, a decrease of the microbial biomass and respiratory activity of the soil affects negatively to the soil enzymatic activity.

The decrease in soil microbial biomass and activity can be also due to the high Na content of BV (Tejada et al., 2006c).

For TP and TP + BV amended soils, the organic amendment increased soil microbial biomass and soil respiration. According to Tejada and Gonzalez (2006a) and Tejada et al. (2006c), the general increase in biomass C observed can be attributed to a positive effect of these organic materials in the soil physical properties and also, to the incorporation of easily degradable materials, which stimulate the autochthonous microbial activity of the soil as well as the incorporation of exogenous microorganisms (Schaffers, 2000; Tejada and Gonzalez, 2006a). Soil microbial respiration, measured through carbon dioxide production is a direct indicator of microbial activity, and indirectly reflects the availability of organic material (Gomez et al., 2001; Tejada and Gonzalez, 2006a).

Soil microbial biomass increase affects positively soil respiration and enzymatic activities.

Dehydrogenase activity typically occurs in all intact, viable microbial cells. Thus, its measurement is usually related to the presence of viable microorganisms and their oxidative capability (Trevors, 1984). According to Rao and Pathak (1996), the incorporation of organic amendments to soil stimulate dehydrogenase activity because the added material may contain intra- and extracellular enzymes and may also stimulate microbial activity in the soil.

Measurement of soil hydrolases provides an early indication of changes in soil fertility since they are related to the mineralization of such important nutrient elements such as C, N, P and S (Ceccanti et al., 1994). Urease catalyses the hydrolysis of urea to carbon dioxide and ammonium, and it is widely distributed in microorganisms, plants and animals (Nannipieri et al., 2002). The stimulation of this

enzymatic activity in TP and TP + BV treatments with respect to the non-amended soil suggests that these amendments do not contain compounds toxic for this enzyme. They increased soil microbial growth due to the substrates added, and the added microbial cells and/or enzymes, which counteract any inhibitory effect of the toxic compounds. The observed stimulation of urease activity was higher in the TP + BV treatments than in the TP treatment, probably due to the higher microbial biomass produced in response.

β -Glucosidase activity reflects the state of the organic matter and the processes occurring therein (García et al., 1994). The higher β -glucosidase activity in organic amended soils can be explained by the positive effect of the organic amendment on the activity of this enzyme, probably due to the higher microbial biomass produced in response (Tejada et al., 2006c).

Soil phosphatase and arylsulfatase activities were higher in the TP + BV amended soils than in TP amended soils. The high activity detected in the amended soils suggests either the existence in the organic wastes of phosphorus and sulphate compounds that can act as substrate for the enzyme, or the existence of microbial populations which need inorganic phosphorus or sulphur for their own development, stimulating the enzyme synthesis.

As regards the results of the varimax rotated factor matrix, the studied properties were grouped into two factors; one of them containing all the physical and chemical properties (which need a long time to affect the soil properties), and the other containing all the biological properties, which respond rapidly and sensitively. These results are in agreement with García et al. (2000), who found that soil biological and biochemical parameters are more sensitive indicators of changes occurring in the soil than physical or chemical parameters. Due to their sensitivity, these properties provide rapid and accurate information on changes in soil quality.

4.3. Plant cover

Since soil enzymatic activities are responsible for important cycles such as C, N, P and S, plant cover increased significantly when TP and TP + BV treatments were applied to the soil. The density of plant cover at the fourth year was higher than that of the third, second and first year, respectively, due to the residual effect of the organic matter of each organic waste after its application in the third, second and first experimental seasons.

These results are very important, principally in arid zones where the risk of desertification and soil loss is a real problem (García et al., 1994).

The decrease in plant cover when soil is amended with BV can be explained by the negative effect of this amendment rich in Na^+ on soil structure and the toxic effect of Na^+ on plants and microorganisms. Since soil enzymatic activities decreased progressively during the experimental period, the density of plant cover at the fourth year was

lower than that of the third, second and first year, respectively.

5. Conclusions

The application of fresh beet vinasse under dryland conditions caused a decrease in soil physical, chemical and biological properties and plant cover density, in spite of having a high organic matter content. Perhaps the increase in monovalent cations (Na^+ , particularly) is responsible for this behaviour. The application of green manure constituted by leguminous plants (*T. pratense* L.) had a positive effect in soil physical, chemical and biological properties, and therefore this organic waste may be considered a good strategy for recovering semiarid areas. When BV was co-composted with the green manure previously mentioned principally at a rate of 2 (green manure):1 (beet vinasse), an increase in soil physical and biological properties was obtained. This organic treatment also favours the appearance of spontaneous vegetation, which will protect the soil and will contribute to its restoration. Consequently, the addition of beet vinasse co-composted with this green manure may be considered a good strategy for recovering semiarid areas.

References

- Aylmore, L.A.G., Sills, I.D., 1982. Characterization of soil structure and stability using modulus of rupture – exchangeable sodium percentage relationships. *Austr. J. Soil Res.* 20, 213–224.
- Baize, D., 1988. Guide des analyses courantes en pédologie. INRA, Paris.
- Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124, 3–22.
- Canfield, R.H., 1941. Application of the line intercept methods in sampling range vegetation. *J. For.* 39, 384–388.
- Ceccanti, B., Pesarosa, B., Gallardo-Lancho, F.J., Masciandro, G., 1994. Bio-tests as markers of soil utilization and fertility. *Geomicrobiol. J.* 11, 309–316.
- Chaney, K., Swift, R.S., 1984. The influence of organic matter on aggregate stability in some British soils. *J. Soil Sci.* 35, 415–420.
- Díaz, M.J., Madejon, E., López, F., López, R., Cabrera, F., 2002. Composting of vinasse and cotton gin waste by using two different systems. *Resources, Conservation and Recycling* 34, 235–248.
- Dick, W.A., Tabatabai, M.A., 1993. Significance and potential uses of soil enzymes. In: Blain, F.J. (Ed.), *Soil microbial ecology: Application in agricultural and environmental management*. Marcel Dekker, New York, pp. 95–127.
- Doran, J.W., Fraser, D.G., Culik, M.N., Liebhardt, W.C., 1988. Influence of alternative and conventional agricultural management on soil microbial process and nitrogen availability. *Am. J. Alternative Agric.* 2, 99–106.
- Eriksen, J., 2005. Gross sulphur mineralization-immobilization turnover in soil amended with plant residues. *Soil Biol. Biochem.* 37, 2216–2224.
- García, C., Hernandez, T., Costa, F., Ceccanti, B., Masciandro, G., 1993. The dehydrogenase activity of soils and ecological marker in processes of perturbed system regeneration. In: XI International Symposium Environmental Biogeochemistry, Salamanca, Spain, pp. 89–100.
- García, C., Hernández, T., Costa, F., Ceccanti, B., 1994. Biochemical parameters in soils regenerated by the addition of organic wastes. *Waste Managem. Res.* 12, 457–466.
- García, C., Hernandez, T., Pascual, J.A., Moreno, J.L., Ros, M., 2000. Microbial activity in soils of SE Spain exposed to degradation and desertification processes. Strategies for their rehabilitation. In: C.

- Garcia, M.T. Hernandez (Eds.), *Research and Perspectives of Soil Enzymology in Spain*, CEBAS-CSIC, Spain. pp. 93–143.
- Golchin, A., Clarke, P., Oades, J.M., Skejtmstad, J.O., 1995. The effects of cultivation on the composition of organic matter and structural stability of soils. *Austr. J. Soil Res.* 33, 975–993.
- Gomez, E., Ferrears, L., Toresani, S., Ausilio, A., Bisaro, V., 2001. Changes in some soil properties in a Vertic Argiudoll under short-term conservation tillage. *Soil Till. Res.* 61, 179–186.
- Gitian, F., Carballas, T., 1976. *Técnicas de análisis de suelos*. Picro Sacro (Ed.), Santiago de Compostela, España.
- Hénin, S., Monnier, G., 1956. Evaluation de la stabilité de la structure du sol. In: VI Congr. Sci. Du Sol. Paris, pp. 49–52.
- Jastrow, J.D., Miller, R.M., 1991. Methods for assessing the effects of biota on soil structure. *Agric., Ecosyst. Environ.* 34, 279–303.
- Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol. Fert. Soils* 6, 68–72.
- Kay, B.D., da Silva, A.P., Baldock, J.A., 1997. Sensitivity of soil structure to changes in organic carbon content: Predictions using pedotransfer functions. *Can. J. Soil Sci.* 77, 655–667.
- Kononova, M.M., 1966. *Soil organic matter*, second ed. Pergamon Press, Oxford.
- Madejon, E., Lopez, R., Murillo, J.M., Cabrera, F., 2001. Agricultural use of three (sugar-beet) vinasse composts: effect on crops and chemical properties of a Cambisol soil in the Guadalquivir river valley (SW Spain). *Agric., Ecosyst. Environ.* 84, 53–65.
- Mamedov, A.I., Shainberg, I., Levy, G.J., 2002. Wetting rate and sodicity effects on interrill erosion from semi-arid Israeli soils. *Soil Till. Res.* 68, 121–132.
- MAPA. 1986. *Métodos oficiales de análisis*. Ministerio de Agricultura, Pesca y Alimentación 1, 221–285.
- Masciandaro, G., Ceccanti, B., Garcia, C., 1994. Anaerobic digestion of straw and piggery waste waters. II. Optimization of the process. *Agrochimica* 38, 195–203.
- McKinley, V.L., Vestal, J.R., Eralp, A.E., 1985. Microbial activity in composting (I). *Biocycle* 26, 39–43.
- Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns, R.G., Dick, R.P. (Eds.), *Enzymes in the Environment. Activity Ecology and Applications*. Marcel Dekker, New York, pp. 1–33.
- Paul, E.A., Clark, F.E., 1989. Reduction and transport of nitrate. In *Soil microbiology and biochemistry*, vol. 9. Academic Press, New York, pp. 81–85.
- Pernes-Debuyser, A., Tessier, D., 2004. Soil physical properties affected by long-term fertilization. *Eur. J. Soil Sci.* 55, 505–512.
- Piccolo, A., Mbagwu, J.S.C., 1990. Effects of different organic wastes amendments on soil microaggregates stability and molecular sizes of humic sizes. *Plant Soil* 123, 27–37.
- Piccolo, A., Pietramellara, G., Mbagwu, J.S.C., 1997. Use of humic substances as soil conditioners to increase aggregate stability. *Geoderma* 75, 267–277.
- Rao, D.L.N., Pathak, H., 1996. Ameliorative influence of organic matter on the biological activity of salt affected soils. *Arid Soil Research Rehabilitation* 10, 311–319.
- Richards, L.A., 1954. *Diagnosis and improvement of saline and alkali soils*. USA Department of Agriculture Handbook, vol. 60. USDA Government Printing Office, Washington, DC.
- Ros, M., Hernández, M.T., García, C., 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biol. Biochem.* 35, 463–469.
- Schaffers, A.P., 2000. In situ annual nitrogen mineralization predicted by simple soil properties and short-period field incubation. *Plant and Soil* 221, 205–219.
- Sims, J.R., Haby, V.A., 1971. Simplified colorimetric determination of soil organic matter. *Soil Sci.* 112, 137–141.
- Soil Survey Staff, 1987. *Keys to Soil Taxonomy*. SMSS Technical Monograph No. 6. third ed., New York.
- Spaccini, R., Mbagwu, J.S.C., Igwe, C.A., Conte, P., Piccolo, A., 2004. Carbohydrates and aggregation in lowland soils of Nigeria as influenced by organic inputs. *Soil Till. Res.* 75, 161–172.
- Soil Survey of England and Wales. 1982. *Soil Survey Laboratory methods*. Technical Monograph 6. SSEW, Harpenden, UK.
- Statistical Graphics Corporation. 1991. *Statgraphics 5.0*. Statistical Graphics System. Educational Institution Edition. USA. p. 105.
- Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenol phosphate in assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.
- Tabatabai, M.A., Bremner, J.M., 1970. Arylsulfatase activity of soils. *Soil Sci. Soc. Am. Proc.* 34, 225–229.
- Tate III, R.L., 2002. Microbiology and enzymology of carbon and nitrogen cycling. In: Burns, R.G., Dick, R.P. (Eds.), *Enzymes in the environment*. Marcel Dekker, Inc. New York, pp. 227–248.
- Tejada, M., Gonzalez, J.L., 2004. Effects of application of a byproduct of the two-step olive oil mill process on maize yield. *Agron. J.* 96, 692–699.
- Tejada, M., Gonzalez, J.L., 2005. Beet vinasse applied to wheat under dryland conditions affects soil properties and yield. *Eur. J. Agron.* 23, 336–347.
- Tejada, M., Gonzalez, J.L., 2006a. Relationships between erodibility and erosion in a soil treated with two organic amendments. *Soil Till. Res.* 91, 186–198.
- Tejada, M., Hernández, M.T., García, C., 2006b. Application of two organic amendments on soil restoration: effects on the soil biological properties. *J. Environ. Qual.* 35, 1010–1017.
- Tejada, M., García, C., Gonzalez, J.L., Hernández, M.T., 2006c. Organic amendment based on fresh and composted beet vinasse: influence on physical, chemical and biological properties and wheat yield. *Soil Sci. Soc. Am. J.* 70, 900–908.
- Trevors, J.T., 1984. Dehydrogenase in soil: a comparison between the INT and TTC assay. *Soil Biol. Biochem.* 16, 673–674.
- Tucker, B.M., 1954. The determination of exchangeable calcium and magnesium in carbonate soils. *Austr. J. Agric. Res.* 5, 705–715.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707.
- Van Veen, J.A., Kluikman, P.J., 1990. Soil structural aspects of decomposition of organic matter by micro-organisms. *Biogeochemistry* 11, 213–233.
- Walker, R.F., 2003. Comparison of organic and chemical soil amendments used in the reforestation of a harsh Sierra Nevada site. *Rest. Ecol.* 11, 446–474.
- Whalen, J.K., Hu, Q., Liu, A., 2003. Compost application increase water-stable aggregates in conventional and no-tillage systems. *Soil Sci. Soc. Am. J.* 67, 1842–1847.
- Yeomans, J.C., Bremner, J.M., 1988. A rapid and precise method for routine determination of organic carbon in soil. *Comm. Soil Sci. Plant Anal.* 19, 1467–1476.
- Zibilske, L.M., 1994. Carbon mineralization. In: Weaver, R.W. (Ed.), *Methods of soil analysis. Part 2. Microbiological and biochemical properties*, SSSA Book Ser, vol. 5. SSSA, Madison, WI, pp. 835–863.